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USE OF AVIAN ANTIBODIES

Field of invention

This invention relates to a method for prophylaxis or therapy of enteric infections in newborn infants, more specifically to a method for prophylaxis or therapy of infections caused by Enterobacter cloaceae.

Background of the invention

All kinds of infections are severe and sometimes life threatening in newborn infants – especially in those who are born prematurely. An effective immune system is of outmost importance to fight infections. The production of immunoglobuline is very immature in newborn infants. Thus, they have to rely solely on immunoglobulines transported to them from their mother via placenta. In infants born prematurely (before the 32nd week of pregnancy) these immunoglobulines are nearly totally absent.

The only treatment of infections in the newborn infants that exists today is the use of antibiotics against bacterial infections and antimycotics against fungal infections. Such treatments have many drawbacks. Antibiotics and antimycotics are very often toxic, e.g. detrimental for the sense of hearing, for the neurologic system, for the kidneys and for the bone marrow, and the newborn infant is more susceptible to these toxic effects than older children and adults. Antibiotics may even cause alternative infections, especially fungal infections, which are more severe than the original infection. The most alarming drawback regarding antibiotics and antimycotics is, however, that several bacteria and fungi very rapidly are becoming resistant against these antimicrobial drugs. Thus, repetitive use of any antimicrobial agents is undesirable.

The natural response by humans to any infection is the production of antibodies of the IgG-class directed to systemic infections and secretory IgA directed to infections of the mucus membrane, including the oral cavity, the digestive tract and genitourinary tract. As the immunsystem is not mature to produce antibodies in the newborn, the ability to use an extraneous source of immunglobulines is a desirable objective in the case of treatment and prophylaxis in newborn infants.

Conventional extraneous sources of bulk polyclonal antibody are derived from serum of mammals after having immunised the mammal with a specific microbe. These polyclonal

antibodies are antigenic in themselves and are therefore unsuitable for long term application in humans. The raise of anti-IgG antibodies will cause loss of activity of the IgG. Orally administered IgY does not raise any anti-IgY antibodies. Thus IgY will not loose activity even after having been used for a long time. The production of IgG antibodies requires a large number of animals, which have to be bled repeatedly. Thus there are also ethical and animal protection objections to the use of IgG.

Summary of the invention

In view of the drawbacks associated with prior art methods for treatment and prophylaxis of enteric infections in newborn infants, there is a need for a new and improved method for treatment and prophylaxis of such infections.

The present invention relates to a novel method for treatment and prophylaxis of such enteric infections, which is both safe and effective. This is achieved by using IgY directed against enteric bacteria, and in particular against Enterobacter cloacae, which have been obtained from the egg yolk of birds, that have been hyperimmunised with an antigen (=microbe) in order to stimulate the production of immunoglobulines (IgY) against this microbe.

The conventional way of treating newborn infants with enteric infections has previously been by using antibiotics. This way has several disadvantages as mentioned above. The applicants' intensive studies of problems related to the gastrointestinal tract have led them to the surprising discovery that, due to the unique properties of newborn infants both regarding their gastrointestinal tract and immune system, a very efficient treatment of enteric infections in newborn infants can be achieved by using avian IgY directed against enteric bacteria. No one has previously considered this possibility.

The present invention also relates to a pharmaceutical product from eggs of birds containing immunoglobuline or a fraction thereof, which can be combined with other preparations or pharmaceuticals for simultaneous, separate or sequential use in the prophylaxis or therapy of gastrointestinal infections in newborn infants.

The present invention relates to prophylaxis or therapy of all kind of infections in newborn infants, prematurely born infants, and infants having an immature immune system. The infection can be any infection caused by an antigen such as bacterium,

virus, fungus or parasite, that is infections in general, preferably bacterial infections, more preferably enteric infections and most preferably infections caused by Enterobacter cloacae.

Detailed description if the invention

The human infant in the first six months of life is particularly vulnerable to diarrhoea. The vulnerability to diarrhoeal processes is associated with the state of development of the gastrointestinal tract, not only of its digestive and absorptive processes but also of its unique and elaborate local defence system, consisting of both immune and non-immune elements. Protection from enteric infection, and from sensitisation to food antigen is a function of the integration of gastric acid and biliary secretion, intestinal motor activity and local immune mechanisms. Development of these functions is controlled by a species-specific programme, in which the potential for accelerated development is very limited.

A newborn infant differs in many essential ways from older children and adults. As mentioned above, the immune system, especially regarding the B-cell-derived immunoglobulines, is very immature in the newborn infant's, and they have to rely on immunoglobulines that have been transported to them from their mother via placenta. Immunoglobulines are nearly totally absent in infants born prematurely before the 32nd week of pregnancy.

The present invention is based on the discovery that anti-Enterobacter IgY has an unexpectedly good capacity to cure prematurely born newborn infants who have a very poor capacity to fight infections due to their immature immune system. The amount of gastric acid, which is an important factor in the protection against bacteria in the intestinal canal, is low in newborn infants. The unexpectedly good effect of the present invention is probably due to the relatively high pH in the stomach of the newborns, i.e. approximately 2-4. The normal pH of the stomach, i.e. approximately 1, would otherwise probably have inactivated the IgY.

IgY is the chicken antibody that corresponds to mammalian IgG. IgY consists of two light chains and two heavy chains and has a molecular weight of approximately 180 000 Da. IgY is actively transported from the hen to the egg and the egg yolk, which thus contains high concentrations of IgY.

One egg yolk contains around 100 - 200 mg of IgY antibodies. Most humans regularly consume ½ - 1½ egg per day and have achieved tolerance against proteins from eggs (including the immunoglobuline (IgY)). These patients will not get any allergic reaction when treated with IgY. Thus, there is no risk for an allergic response when treating these patients with IgY. However, patients with known egg allergy should not be treated with IgY. A dose in the order of 2 mg IgY would probably suffice to achieve the desired prophylactic or curing effects.

Hens, which have been immunised with microbes, respond by producing specific, polyclonal antibodies against the microbe. The antibodies can be purified from the egg yolk. Several in vitro studies show that bacterial, viral and fungal infections can be prevented with IgY. Many studies have also shown that peroral administration of specific IgY is used successfully to treat bacterial and viral infections in animals.

Compared to mammalian polyclonal antibodies IgY reacts with different epitopes on the antigen than the mammalian antibodies do. This gives access to a different antibody repertoire than the mammalian antibody. The mode of action of the specific antibody is related to the number of organisms present at a given moment. It will be appreciated that there is a direct molecular correlation between antibody entities attaching to each microbe and the numbers of microbes present. The dose level will also be related to the total surface area of affected tissue and biological parameters which affect "wash out" ratios.

When the immunoglobuline is to be administered by the oral route, it will preferably contain a buffering agent to prevent deactivation at low pH-values which can optionally be administered in the form of a nutritional complement.

IgY antibodies also have biochemical properties that make them advantageous over IgG for peroral immunotherapy: They neither activate the human complement system nor react with rheumatoid factors, human anti-mouse IgG antibodies (HAMA) or human Fc-receptors. Those are all well-known cell activators and mediators of inflammation.

As IgY is a normal dietary component there is no risk of toxic reactions in the patient, except for those who have a known allergy to eggs.

Egg immunoglobuline is classified as avian IgY which is similar to mammalian secretory IgA, and therefore a natural part of the mucus epithelial environment.

The object according to the invention is to provide a pharmaceutical composition from eggs of birds comprising immunoglobuline or a fraction thereof for use in the prophylaxis or therapy of infections in the newborn infant.

As indicated above, the infections in newborn infants can be attributed to a multiplicity of factors, including long-term exposure to antibiotics, which disrupt the normal balance of the intestinal micro-flora. The preparation will be designed to reinstitute the normal balance of the microflora of the newborn infants. The formulations according to the present invention can be used as an alternative or supplement to such treatments.

According to a basic embodiment of the present invention, by mixing the IgY according to the present invention with any pharmaceutically acceptable carrier or diluent, a pharmaceutical composition or medicament is obtained. The medicament containing IgY can be formulated as a freeze dried or lyophilised powder, a solution, a lozenge, a tablet or as a capsule. This pharmaceutical medicament is preferably administered in a form suitable for oral administration. together with any other pharmaceutically acceptable carrier or diluent.

In another embodiment the pharmaceutical medicament according to the present invention is formulated as a controlled or sustained release formulation.

According to another embodiment of the present invention, the IgY can be administered without any conventional diluent or recipient in a nutritional agent such as human breast milk or a substitute therefor.

One embodiment of the present invention relates to a method of prophylaxis or treatment of newborn infants having a pH in their stomachs above 1,5, particularly between 1,5 and 4. This method comprises administrating IgY, that originates from an egg of a bird hyperimmunised with a microbe, to the infant. The method particularly relates to prophylaxis or treatment of prematurely born infants, and more particularly to infants having a weight below 2500g.

In the present application different terms have been used which will be defined below.

By the terms "immunoglobuline" or "fragment of an immunoglobuline" is meant an antibody, or antibody fragment, or antibody precursor capable of binding to a specific microbe or fragment thereof so as to render it non-pathogenic.

The pharmaceutical product according to the present invention can also be used in conjunction with, or include, an antimicrobial agent of the kind used in conventional therapy of infections of the newborn infants.

Examples

Preparation of anti-Enterobacter cloacae IgY immunoglobuline.

A suspension of formalin-killed Enterobacter cloacae was washed in saline and freeze-dried in ampoules; 2.0 x 108 bacterial cells per ampoule. Twice a week, inoculations were carried out intramuscularly in domestic hens, using 1.0 ml purified water to resuspend each ampoule. Yolks of eggs collected from hyper-immunised hens were assayed to determine peak antibody titre using an ELISA (enzyme linked immunosorbent assay) specific for Enterobacter cloacae IgY.

After 3 to 4 weeks, when peak titre had been achieved, egg yolks were harvested by separation from the egg white and removing the contents of the yolk sac using a hypodermic syringe. The immunoglobuline fraction was purified using an industrial standard of supercritical CO_2-equipment to dissolve the lipid. Leaving the proteinaceous polyclonal immunoglobuline in a purified state. The immunoglobuline fraction was diluted using distilled water to a concentration of 10 mg/ml and lyophilised in trays. This solution was used to evaluate the prophylactic potential of the anti-Enterobacter IgY indicated below in the example *Treatment of newborn infants with anti-Enterobacter IgY.*

Preparation of Enterobacter cloacae

Enterobacter cloacae isolated from faeces of infected newborns was used in an in vitro experiment to demonstrate the prophylactic potential of egg immunoglobuline isolated from domestic hens hyper-immunised with Enterobacter cloacae antigen.

The bacteria were grown in 500-ml flasks containing 100 ml of 2% glucose, 0.15% yeast nitrogen base, 0.5% ammonium sulphate supplemented, where necessary, with amino acids. The flasks were shaken at 200 r.p.m. in a rotary incubator at 37°C for 24 hours.

Cell adhesion assay

Adhesion to mucus epithelium cells is considered to be the primary stage in infection. In this example epithelial cells and pseudomonas aeruginosa (as representative for bacteria) were used to evaluate adhesion.

Fresh cells cultured for 24 hour were washed by centrifugation in PBS, re-suspended in PBS and mixed with medium containing either IgY of eggs from hens immunised with pseudomonas or IgY from non-immunised hens at a ratio of 100:1 or no IgY at all and incubated at 37°C for 2 hours. After incubation, the culture was filtered through a 45gm filter to remove any unadhered cells, washed in PBS and re-suspended.

The adherence was evaluated by microscopic examination at 400 magnifications using a stage micrometer grid to facilitate accurate counting. Adherence was expressed as a percentage of cells with visibly adhering pseudomonas aeruginosa bacteria.

The results showed that the adhesion of *P. aeruginosa* to epithelial cells was reduced by more than 50% in the case of bacteria treated with immunoglobuline from immunised hens, when compared with both untreated bacteria and bacteria treated with extract of normal egg.

The above in vitro experiments demonstrate that purified immunoglobuline fractions extracted from the yolk sac of eggs laid by hens previously hyperimmunised with P. aeruginosa antigen inhibit epithelial cell adhesion. These experiments enables one to conclude that specific egg immunoglobuline can be used in the prophylaxis or therapy of infections in the newborn infants.

Treatment of newborn infants with anti-Enterobacter IgY.

Infections caused by Enterobacter chloacae are extremely dangerous for prematurely born infants. Half of the infants with a birth weight of 2500 g and below, who gets an infection with Enterobacter chloacae, will have severe neurologic sequel or even die.

In the experiment anti-Enterobacter IgY was used to treat prematurely born infants of less than 2500g, who have been infected by Enterobacter chloacea. Twelve infants were daily given IgY with a high specific titre against Enterobacter in human breast milk. The treatment started as soon as a culture from stools or blood had been positive for Enterobacter chloacae. In eleven of these patients Enterobacter chloacae was nearly immediately eradicated from their stools. Only one patient, who already had a bacteraemia with Enterobacter before the treatment started, continued to have stool and blood cultures positive for Enterobacter for nearly a month. None of the patients died and none of them got any sequel.

The intention is not limited to the embodiments described above which may be modified and/or varied without departing from the scope of the invention.

Claims

1. IgY that originates from an egg of a bird hyperimmunised with a microbe for use in prophylaxis or treatment of enteric infection in newborn infants.

2. IgY according to claim 1, wherein the microbe is a bacterium, virus, fungus or parasite.

3. IgY according to any of the preceding claims, wherein the infection is a bacterial infection.

4. IgY according to any of the preceding claims, wherein the microbe is Enterobacter cloacae.

5. Pharmaceutical composition, comprising the IgY according to any of the claims 1-4, wherein the IgY is formulated as a freeze dried or lyophilised powder, a solution, a lozenge, a tablet or as a capsule together with any other pharmaceutically acceptable carrier or diluent.

6. Pharmaceutical composition according to claim 5, further containing a nutritional agent.

7. Pharmaceutical composition according to claim 5 or 6, wherein the nutritional agent is human breast milk or a substitute therefor.

8. Method of prophylaxis or treatment of enteric infections in newborn infants, comprising the step of:
administrating a composition comprising IgY that originates from an egg of a bird hyperimmunised with a microbe to a newborn infant.

9. Method according to claim 8, wherein the microbe is a bacterium, virus, fungus or parasite.

10. Method according to claim 8, wherein the infection is a bacterial infection.

11. Method according to claim 8; wherein the microbe is Enterobacter cloacae.

12. Method according to claim 8, further comprising formulating the composition as a freeze dried or lyophilised powder, a solution, a lozenge, a tablet or as a capsule or administering it together with any other pharmaceutically acceptable carrier or diluent.

13. Method according to claim 8, further comprising administration of a nutritional agent.

14. Method according to claim 9, wherein the nutritional agent is human breast milk or a substitute therefor.

15. Method according to claim 8, wherein the composition is administered to newborn infants having an immature immune system.

16. Method according to claim 8, wherein the composition is administered to newborn infants having a weight below 2500g.

17. Method according to claim 8, wherein the composition is administered to prematurely born infants.

18. Method according to claim 8, wherein the composition is administered to newborn infants having a pH above 1,5.

19. Method according to claim 8, wherein the composition is administered to newborn infants having pH between 1,5 and 4.

20. Use of IgY that originates from an egg of a bird hyperimmunised with a microbe for the manufacture of a pharmaceutical medicament for use in prophylaxis or treatment of enteric infection in newborn infants

Abstract

The present invention relates to a novel method for treatment and prophylaxis of infections, especially enteric infections, in newborns, which is both safe and effective. This is achieved by using IgY directed against microbes, in particular against Enterobacter cloacae, which have been obtained by hyperimmunising birds with an antigen (microbe) in order to stimulate the production of immunoglobulines (IgY) against such microbe. The present invention also relates to a pharmaceutical product from eggs of birds containing immunoglobuline or a fragment thereof, which can be combined with other preparations or pharmaceuticals for simultaneous, separate or sequential use in the prophylaxis or therapy of gastrointestinal infections in newborn infants.

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